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The Treatment of Possible Severe Infection in Infants: An Open Randomized Safety Trial of Parenteral Benzylpenicillin and Gentamicin Versus Ceftriaxone in Infants <60 days of Age in Malawi

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The authors declare no conflicts of interest

Abbreviated title: Pen/Gent or Ceftriaxone for Sepsis in Malawian Neonates

Running head: Antibiotic Choices for Neonatal Sepsis in Malawi

Keywords: neonatal sepsis, ceftriaxone, adverse events, outcome.

Background: The World Health Organization recommends benzylpenicillin and gentamicin as antimicrobial treatment of infants with sepsis in low income settings (LICs), and ceftriaxone or cefotaxime as an alternative. In a meta-analysis from 13 LICs, *Staphylococcus aureus*, Klebsiella *spp*. and *E.coli* accounted for 55% of infants with sepsis. In a review of bacterial meningitis, resistance to third generation cephalosporins was >50% of all isolates, and 44% of Gram-negative isolates were gentamicin resistant. However, ceftriaxone may cause neonatal jaundice and gentamicin may cause deafness. Therefore, we compared parenteral benzylpenicillin plus gentamicin to ceftriaxone as first line treatment, assessing outcome and adverse events.

Methods This was an open randomized trial carried out in the Queen Elizabeth Central Hospital, Blantyre, Malawi from 2010 to 2013. Infants < 60 days of age with possible severe sepsis received either benzylpenicillin and gentamicin or ceftriaxone. Adverse events and outcomes were recorded until 6 months post discharge.

Results: 348 infants were included in analyses. Outcome in the benzylpenicillin and gentamicin or ceftriaxone groups was similar; deaths were 13.7% and 16.5% and sequelae 14.5% and 11.2% respectively. More infants in the penicillin/gentamicin group required phototherapy: 15% v 5%, p=0.03. Thirteen (6%) survivors had bilateral hearing loss. There was no difference between the treatment groups. By 6 months post discharge 11 more infants had died and 17 more children were found to have sequelae.

Conclusions Ceftriaxone and gentamicin are safe for infants in our setting. Infants should receive long term follow up as many poor outcomes occurred after hospital discharge.

Introduction

In an open, randomized trial of Malawian infants <60 days of age with possible severe bacterial infection, we compared parenteral benzylpenicillin plus gentamicin to ceftriaxone as first line treatment, assessing outcome and adverse events.

Background

Clinically suspected possible severe bacterial infections (pSBIs) are common in low-income settings, especially in the first month of life when mortality and morbidity are high.[1] Early and appropriate therapy are critical to a good outcome. Antimicrobial therapy is guided by World Health Organisation (WHO) recommendations: first line therapy with parenteral benzylpenicillin and gentamicin; second line treatment with cefotaxime or ceftriaxone.[2] Gentamicin has a potential for toxicity, especially hearing loss, but methods of monitoring blood concentrations of the drug are rarely available. There are no studies comparing the two regimens for efficacy, adverse events and outcome. Possible severe bacterial infection includes severe pneumonia, sepsis and meningitis. The WHO Young Infants sepsis study group reported that in a multicenter study in low income countries (LIC) in Asia and Africa, the three most common causes of pSBI found were Gram negative enteric bacteria, Group B Streptococcus (GBS) and S*treptococcus pneumoniae.*[3] In a meta-analysis of reports from 13 low income settings, *Staphylococcus aureus*, Klebsiella *spp*. and *E.coli* accounted for 55% (39-70%) of culture positive sepsis in all infants;[4] findings confirmed in a review of 21 studies, published after 2000, of neonatal invasive bacteremia in low income settings, 10 of which were in sub Saharan settings.[5] In a six-country review of bacterial meningitis, resistance to second and third generation cephalosporins was present in >50% of all isolates, and 44% of Gram-negative isolates were gentamicin-resistant.[6]

Early onset sepsis (<7 days) is often associated with risk factors in the mother and /or delivery and the causative agents are GBS, *S. aureus* and Gram negative bacteria such as *E. coli*. Late onset infections (7-60 days) are commonly caused by bacteria such as *S. pneumoniae*, *S. aureus*, *Klebsiella pneumoniae* and also GBS.[2]

In high-income settings, first line antimicrobial treatment is usually benzylpenicillin or ampicillin and gentamicin for non-meningitis cases, and cefotaxime with ampicillin for meningitis or as second line therapy. The ampicillin is to cover *Listeria monocytogenes* infections.[7] Ceftriaxone has been avoided in infants because of perceived safety issues, especially in infants who are jaundiced or hypoalbuminaemic,[8] because ceftriaxone can cause biliary sludging, although this is reversed when treatment ceases and has no persisting sequelae.[8-11] Ceftriaxone can form ceftriaxone-calcium complexes if given within 48 hours of a calcium-containing intravenous (IV) infusion. These complexes precipitate in IV fluid lines, the lungs and the kidneys, sometimes with fatal results.[12-14] Some national guidelines advise against using ceftriaxone in premature babies until they attain the gestational age of 41 weeks.[15] Ceftriaxone is still the drug of choice in neonatal gonorrheal ophthalmitis.[16]

Except in inflamed meninges, gentamicin has poor CNS penetration, achieves rather poor CSF levels and does not penetrate well into cells. The therapeutic range is narrow and gentamicin may control but fail to eradicate Gram negative infections.[17] In Blantyre *Listeria monocytogenes* is exceptionally rare. This may be because a typical Malawian diet does not include unpasteurized dairy products or salads. Surrounding countries such as Kenya, South Africa and Zimbabwe report similar causes of pSBI as Malawi.[18-20]

Cephalosporins are bactericidal antibiotics and although CNS penetration is modest, higher doses safely achieve therapeutic CSF drug levels. In Malawi benzylpenicillin is appropriate for GBS

infections and for most *S. pneumoniae* infections.[21,22] *Klebsiella pneumoniae* and many other Gram negative bacteria are increasingly resistant to gentamicin.[23,24]

The recommended WHO first line therapy may be inadequate empirical therapy where Gram negative bacteria account for half or more of all cases of SBI in infants <2 months of age. For this reason, we compared benzylpenicillin and gentamicin to ceftriaxone as first line treatment for pSBI in infants, and monitored for safety, especially jaundice, during therapy.

Methods

This was an open randomized trial carried out in the pediatric department of the Queen Elizabeth Central Hospital (QECH) in Blantyre, Malawi from March 2010 to February 2013. QECH is an 1100-bedded public government hospital; it is also the main teaching hospital of the Malawi medical school. It serves as the referral hospital for the southern half of the country and also as the district hospital for Blantyre. The children's department admits 28,000 children a year and about 80,000 children are seen annually in the emergency unit.

Inclusion criteria

Children ≤ 2 months of age in whom there was clinical suspicion of severe sepsis, pneumonia or meningitis were eligible for inclusion. Following WHO guidelines, pSBI (including severe pneumonia, sepsis and BM) was suspected in the presence of convulsions, bulging fontanelle, lethargy, coma, poor feeding, irritability, apneic episodes or abnormal cry.[25] In infants <7 days old an extended diagnostic algorithm included fever, agitation, no spontaneous movement, cyanosis, slow capillary refill (<3 secs) and lower chest wall in-drawing.

Enrolment took place after the guardian was fully informed and written consent was given. We recorded demographic, clinical and laboratory findings, including details about the pregnancy and the delivery.

Exclusion criteria

Infants not to be enrolled were: those with clinical severe jaundice (yellow discoloration of the skin extending to the lower limbs); children with known hypersensitivity to any of the three antibiotics and those who had been hospitalized for >72 hours, to avoid enrolling nosocomial infections. Children with previous neurological abnormalities such as hydrocephalus and neural tube defects were not enrolled. Excluded patients received standard treatment of benzylpenicillin and gentamicin.

Endpoints

The primary endpoints were differences in outcome and occurrence of jaundice between the two treatment groups.

Randomization

Randomization was by computer-generated numbers in blocks of ten. Treatment allocations were sealed in consecutively numbered opaque envelopes and opened in numerical order by the recruiting clinician at enrolment. Allocation was to either to benzylpenicillin 50,000 iu/kg 8 hourly IV (100,000 iu 8 hourly IV for BM) and daily gentamicin 6 mg/kg IV (standard smaller doses for low birth weight infants and very premature babies) or ceftriaxone IV 50 -100 mg/kg od (depending on age) for 5-14 days.

Samples on admission.

Laboratory investigations were carried out at the Malawi-Liverpool-Wellcome Trust Clinical Research Programme Laboratories which are externally quality controlled. A blood sample was taken for a full blood count, culture, electrolytes, glucose and an HIV antibody test (Determine®). All infants testing positive by HIV antibody test had a blood sample tested by PCR to identify active HIV infection.

Cerebral spinal fluid (CSF) was taken for biochemistry, microscopy and culture. A positive CSF was one in which a culture was positive or the white cell count was >50 cells/ul with neutrophils forming the greater proportion of the cells.

Clinical monitoring and care

All infants were monitored by study nurses every 2–4 hours and seen at least twice daily by the study team. Most infants had hearing tests and when clinically appropriate an ultrasound scan of the head. Bilirubin levels were measured twice daily with a transcutaneous bilirubinometer (Konica Minolta Drager Air Shields JM 103). MRIs were carried out when their findings might benefit the child.

We provided supportive care according to unit protocols. Calcium is not added to any infusions and serum gentamicin levels are not available.

If the infant deteriorated despite the treatment given, and after discussion with the principal investigator, an appropriate antibiotic could be added to the treatment schedule or a switch made to the antibiotic(s) in the other study arm. If the CSF or blood culture report showed that a child was receiving inappropriate antimicrobial therapy for the bacteria grown, the treatment was changed for a more suitable antibiotic.

Follow-up

Follow-up was at one and six months after hospital discharge when neurological and hearing assessments were done. Age-appropriate hearing tests were carried out by trained nurses using oto-evoked potentials (Echocheck) and distraction tests. The neurological assessment was made by a trained research clinician.

Sample size

To detect a 40% lower case fatality rate in the ceftriaxone group than in the benzylpenicillin and gentamicin group, (ie to reduce the overall case fatality rate of meningitis from 50% to 30%) with a confidence of 90% and power of 80% required 107 infants in each arm (total = 214). To detect a difference in jaundice development in the ceftriaxone group of 18% compared to 8% in the penicillin and gentamicin group, the sample size needed with a confidence of 90% and power of 80% was 158 in each arm, (total = 316). To allow for mortality (much of it early) and loss to follow up, an extra 10% were to be recruited; i.e. 174 to each group: 348 in total.

Statistical Analysis

Statistical analysis was per protocol, done using Stata version 14.0 StataCorp Texas 77845 USA. The difference in means of normally distributed variables was performed using an independent samples t-test. Chi-square tests were used to assess relationship between categorical variables. Univariate logistic regression model was used to assess factors associated with poor outcome to obtain unadjusted odds ratios. Multivariate logistic regression model was also fitted to identify factors that are independently associated with outcome. All statistical tests were 2 tailed. Statistical significance was declared at a value of <0.05. The 95% confidence intervals for the odds ratios were obtained and reported.

Adverse events

Severe adverse events were reported to a data safety management board (DSMB) through the clinical monitor within 48 hours of their occurrence. The main safety endpoint for ceftriaxone was a transcutaneous bilirubin level at which phototherapy would be instituted. This level depended on gestational and postnatal age according to departmental bilirubin level graphs (see tables, Supplemental Digital Content 1 and 2). If levels were reached that required phototherapy, it was commenced and 8 hourly transcutaneous bilirubin levels were measured. If bilirubin

concentrations decreased or remained stable, further doses of ceftriaxone were given and monitoring continued. If bilirubin concentrations increased, no further ceftriaxone was given. Serious events included deaths, jaundice levels at or beyond 'phototherapy' levels, anemia (Hb <6 g/dl) while on therapy and serious adverse drug reactions (rashes, bronchospasm, anaphylactic shock).

Any changes from one antibiotic to another were reported and the reasons for change documented. The study was to be stopped if bilirubin levels requiring a change in antibiotic therapy were found in 30% more of the children receiving ceftriaxone than of those receiving benzylpenicillin and gentamicin.

Ethical considerations

Benzylpenicillin and gentamicin are widely used to treat neonatal infections despite the theoretical complications of renal failure and hearing loss. Ceftriaxone can cause conjugated bilirubinaemia and jaundice without permanent sequelae. Ceftriaxone has been used for several years in many centers across the region as second line treatment for neonatal infections. Nevertheless because of these theoretical complications all infants were monitored closely.

All guardians gave written consent to be enrolled after being fully informed of the study. Permission was granted by the College of Medicine Research and Ethics Committee (COMREC) to undertake the study (P2010/819) and the trial was registered with clinicaltrials.gov (NCT01247909).

Results

From March 2010 to Feb 2013 a total of 351 infants less than 60 days of age were enrolled; one parent withdrew consent before signing and two infants were deemed not to require antibiotics.

The remaining 348 were included in analyses. (Figure 1) Of these, 161 (46.5%) were given gentamicin / benzylpenicillin and 170 received ceftriaxone: 17 received both. Baseline characteristics were similar in the two groups, except for prevalence of clinical jaundice (n= 12; 6.5% in the penicillin v 23; 14% in the ceftriaxone group p=0.02). Table 1

Overall inpatient mortality was 12%; (n= 41) and a further 11 died within six months of discharge (total mortality n=52;15%). Sequelae were found in 4.6% (n =16) at hospital discharge and a further 17 (total =33;12.6%) of 261 survivors at six months after discharge. (Figure 1). Causes of death after discharge could not be verified but six (18.7%) had neurological sequelae following meningitis, four had significant congenital abnormalities, two HIV positive infants had further admissions for probable *Pneumocystis jirovecii* pneumonia (PJP), four had been admitted with severe shock or sepsis and no cause for later death was given.

Of the 348 patients, 54 (15.5%) did not have a lumbar puncture done, 42 (14.3%) of the remaining 294 had positive CSF cultures of which 15 (36.6%) were Group B Streptococcus (GBS) and 6 (14.6 %) were Gram negative bacteria such as *Acinebacter baumanni, E. coli, Klebsiella pneumoniae* (Table 2)

Blood cultures were done in 348 children; 105 (30.1%) were positive; of these 62 (59%) grew coagulase negative staphylococci, 9 (8.5%) were Gram negative bacteria, 15 (14.3%) were GBS and 11 (10.5%) were *Staphylococcus aureus* (Table 2). Overall more children with a positive than a negative CSF or blood culture had a poor outcome (25(44%) v 67(33%) p=0.003 and 21(41%) v 54(30%) p=0.04 respectively (see table, Supplemental Digital Content 3). Coagulase negative staphylococci and alpha hemolytic streptococci may have been contaminants but some of the infants from whom the samples were taken were very ill. Removing these bacteria from analyses made no difference to the findings. The combined outcome by CSF and blood culture

results comparing no growth with growth (137 v 122) was also significant (p= 0.017) (see table, Supplemental Digital Content 3)

Outcomes were similar between the two treatment groups; inpatient mortality was 11.2% in both the benzylpenicillin and gentamicin and the ceftriaxone arms. (Table 2) At six months post discharge, deaths were 13.7% and 16.5% and sequelae in survivors were 14.5% and 11.2%. respectively, (Figure 1).

On multivariate analysis weight on admission, convulsions, not sucking, an oxygen saturation level < 90% and positive blood culture were each associated significantly with mortality and sequelae (Table 3).

More infants in the penicillin/gentamicin group were clinically jaundiced on admission and more eventually required phototherapy: n=19; 15% v 7; 5%, p=0.03. (Table 4). Fifteen infants received phototherapy for 1-2 days, 8 for 3-4 days, 6 for 5-6 days and 4 for 7-11 days. Of the infants receiving >5 days of phototherapy, two were on ceftriaxone and six were on benzylpenicillin; one received both drug treatments.

Thirteen (6%) of 216 children who were tested had bilateral hearing loss; eight (61.5%) also had neurologic sequelae suggesting that the cause was the underlying infection. In four of the 13 infants with bilateral hearing loss a lumbar puncture was not done as the infants were too sick; eight of the remaining nine infants had a positive culture of blood or CSF. There was no significant difference between the treatment groups. (Table 5)

Discussion

In this study more infants had Gram positive than Gram negative infections. When treated with either benzylpenicillin + gentamicin or ceftriaxone, the outcomes in the two treatment groups were similar. This finding resembles the results of a meta-analysis of studies

that used either of these two protocols.[4] In a previous review of CSF results in our own hospital, Swann et al reported that more neonatal cultures were sensitive to ceftriaxone than to benzylpenicillin and gentamicin (99.1% vs 91.8%; p=0.006), especially the Gram-negative isolates (95.1% v 86.0%; p=0.012).[21] A similar review of neonatal blood cultures done over that same period of time showed that 53% of the pathogens were Gram-positive and 47% Gram-negative. The four most common pathogens were *S. aureus*, GBS, *Salmonella* Typhimurium, and *E. coli*.[22] Klebsiella *sp*, Acinebacter *sp* and Enterobacter *sp*, all considered nosocomial infections, accounted for 7.3%, 3.1% and 4.6% of the Gram negative pathogens The results of our study differ because we included all cases of possible severe bacterial infection, as this reflects clinical practice; only 147 (45%) blood or CSF samples grew bacteria of which 62 (42%) were coagulase negative staphylococci that may, or may not, have been contaminants. Even if the coagulase negative staphylococci are excluded we had more Gram positive (n=36/45; 80%) than Gram negative (n= 9/45; 20%) infections. This is probably because there has been a decline in invasive non typhoidal salmonella infections in Malawi over the last decade [26] and we excluded nosocomial infections.

More infants in the benzylpenicillin/gentamicin group developed jaundice than in the ceftriaxone group. Jaundice was caused mainly by the underlying infection; only seven of 24 (30%) infants commenced phototherapy after starting antibiotics. The overall hospital mortality was 42/348 (12.1%) and 4.6% survived with sequelae. Hearing loss was related to the underlying infection and not to the treatment. The outcome was worse in culture positive pSBI than in culture-negative infants (p=0.017) and worse in infants who were HIV infected or exposed than unexposed (p= 0.008).

Forty one children died in hospital but after six months a further 11 had died. Sixteen children were identified in hospital as having sequelae but by six months 17 more children were found to have sequelae. It is clear that all infants with pSBI need follow up to ensure additional supportive care for those who need it as about half who will eventually have sequelae are likely to be missed at the time of hospital discharge.

Conclusions:

Ceftriaxone is safe in infants in our setting – in particular its use was not associated with a higher frequency of jaundice in this study – and hearing was not affected by gentamicin use. In this study, which did not include infants likely to have nosocomial infections, the outcome from pSBI was similar whether infants were treated with benzylpenicillin and gentamicin or with ceftriaxone. Infants with pSBI should be followed up for at least 6 months, as many may develop sequelae that were not detected on hospital discharge.

Acknowledgements

We thank all the families who allowed us to study and care for their infants. We thank the pediatric department for their help in recruiting and caring for the children; N. Kennedy, the clinical monitor and M. Mukaka who gave wise statistical advice. Malawi Liverpool Wellcome Trust Clinical Research Programme is supported by a strategic award from the Wellcome Trust.

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Supplemental Digital content legend

Supplemental Digital Content 1. LEVELS OF BILIRUBIN AT WHICH TO START PHOTOTHERAPY IN TERM INFANTS

Supplemental Digital Content 2. LEVELS OF BILIRUBIN AT WHICH TO START PHOTOTHERAPY IN PREMATURE INFANTS

Supplemental Digital Content 3. CSF and blood culture findings and outcomes in infants with possible severe bacterial infection.

Table 1

Characteristic	Unit of measure	Ceftriaxone N =170	Penicillin/Gentamicin N=161	Total * N = 331	P value
Sex:N (%)	Female	82/167 (49%)	75/157 (48%)	157/324 (48%)	0.83
Known Birth weight	Number (%)	123 (72)	132 (82)	255 (77)	0.05
Admission weight Kgs	Number known (%) Median [IQR]	170 (100) 3.2 [1.9, 4.5]	160 (99) 3.1 [1.9, 4.2]	330 (99.5)	0.49
Age groups: n (%)	≤7 days 8days -30days ≥ 30 days	34 (20) 93 (55) 43 (25)	43 (27) 78 (48) 40 (25)	77 (23) 171(52) 83 (25)	0.33
Mode of delivery n(%)	LSCS[a]/Instrumental SVD Unrecorded	16 (9) 151(89) 3 (2)	10 (6) 150 (93) 1 (1)	26 (8) 301(91) 4 (1)	0.31
Parity: n (%)	Single Twins Unrecorded	152 (89) 16 (9.5) 2 (0.5)	151 (94) 9 (5.5) 1 (0.5)	303(92) 25 (7.5) 3 (0.5)	0.21
Temperature: n (%)	<36.5^{0}C 36.5- 37.5^{0}C >37.5^{0}C Unrecorded	26 (15.5) 106 (62.5) 36 (21.5) 2 (0.5)	20 (12) 98 (61) 41 (26) 2 (1)	46 (14) 204(62) 77 (23) 4 (1)	0.56
Fever days:n (%)	≤1 day 1 - 2 day >2 days Unrecorded	85 (50) 40 (24) 43 (25) 2 (1)	78 (48) 42 (26) 40 (25) 1 (1)	163(49) 82 (25) 83 (25) 3 (1)	0.88
Sucking : n (%)	Yes No Unrecorded	116 (68) 52 (31) 2 (1)	124 (79.5) 36 (22) 1 (0.5)	240(72.5) 88 (26.5) 3 (1)	0.10
Convulsions: n (%)	Yes No Unrecorded	22 (13) 147 (86.5) 1 (0.5)	15 (9.5) 145 (90) 1 (0.5)	37 (11.5) 292(88) 2 (0.5)	0.38
Total bilirubin[b] n (%)	<1mmol/l >1 mmol/l Unrecorded	158 (93.5) 9 (5.5) 3 (1)	138 (86) 21 (13) 2 (1)	296(90) 30 (9) 4 (1)	**0.02**
Difficult breathing: n (%)	No Yes Unrecorded	82 (48) 85 (50) 3 (2)	80 (49.5) 80 (49.5) 1 (0.5)	162(49.5) 165(49.5) 4 (1)	0.91
Cough days: n (%)	≤1 day >1 day Unrecorded	119 (70) 50 (29.5) 1 (0.5)	112 (69.5) 48 (30) 1 (0.5)	231(70) 98 (29) 2 (0.5)	1
Haemoglobin	Number tested (%) Median g/dl{range}	162 (95) 12.5{3.6-39}	148 (92) 12.95{1.1 -22.8}	310(94)	0.26
Oxygen saturation %	≤90% >90% Unrecorded	112 (66) 49 (29) 9 (5)	106 (66) 48 (30) 7 (4)	218(66) 97 (29.5) 16 (4.5)	0.90
Total blood WBC : n(%)	≤5000 cm^3 5 - ≤10,000 cm^3 10 – ≤15,000 cm^3 >15,000cm^3 Unrecorded	16 (9) 49 (29) 52 (31) 44 (26) 9 (5)	18 (11) 38 (24) 44 (27) 47 (29) 14 (9)	34 (10.5) 87 (26) 96 (29) 91 (27.5) 23 (7)	0.65
Malaria Parasites on BF[c] or positive MRDT[d] n(%)	Negative Not done	155 (91) 15 (9)	144 (89) 17 (11)	299(90) 32 (10)	0.71

*excluded 17 cases who received both antibiotic therapies; [a] = lower section Caesarean section; [b] measured transcutanously [c] Blood film; [d]Malaria rapid diagnostic test

Table 1 Baseline characteristics on admission

Table 2

Table 2. CSF and Blood culture findings in neonatal sepsis

	CSF culture results	Blood Culture results
	Number(%)	Number(%)
Not done/missing	53 (15)	7 (2)
No growth*	254 (73)	220 (66)
Group B streptococcus	15 (4)	16 (5)
Coagulase negative staphylococcus	8 (2)	63 (19)
*Streptococcus pneumoniae***	8 (2)	5 (1)
Streptococcus pyogenes	2 (1)	0
Staphylococcus aureus	0	11 (3)
Group D streptococcus	0	1 (0.5)
Alpha haemolytic streptococcus	2 (1)	3 (1)
Gram negative[x]	6 (2)	9 (2.5)
Total	348 (100)	335 (100)

*12 CSFs with no growth on culture had white cell counts (number of cells = 23-clumps in pus) suggestive of meningitis, 3 of these infants had positive blood cultures (1 each of Group B streptococcus, coagulase negative staphylococcus and alpha haemolytic streptococcus, **1 had Gram positive diplococci on Gram stain, but was culture negative)

[x] The Gram negative bacteria were *E. coli* 3, Acinebacter baumanni 2, *salmonella* Typhimurium 1, Enterobacter cloacae 1, Acinebacter lwolfi 1.

All diploccici, micrcci, and bacilli were considered contaminants. Coagulase negative staphylococci and alpha haemolytic streptococci may have been contaminants but some of the infants from whom the samples were taken were very ill.

Table 3 Multivariate analyses of variables affecting outcome

		Outcome		Multivariate	
Variable	Number assessed	Alive, no Sequelae N= 186	Dead or Sequelae N= 91	OR, 95% CI	P value
Gentamicin/Penicillin	134	96 (63)	38 (37)		
Ceftriaxone	143	90 (72)	53 (28)	0.69 (0.34, 1.36)	0.28
CSF culture -ve	200	136 (68)	64 (32)		
CSF culture +ve	36	21 (58)	15 (42)	2.15 (0.89, 5.15)	0.08
Blood culture -ve	181	128 (71)	53 (29)		
Blood culture +ve	93	55 (59)	38 (41)	2.15 (1.07, 4.38)	**0.033**
Weight Kg >2.5	225	162 (72)	63 (28)		
<=2.5	51	24 (47)	27 (53)	2.46 (1.12, 5.42)	**0.024**
HIV -ve	195	134 (69)	61 (31)		
HIV exposed	56	42 (75)	14 (25)	1.15 (0.49,2.58)	
HIV+ve	9	3 (33)	6 (67)	4.30 (0.84,25.2)	0.22
Convulsion none	248	175 (71)	73 (29)		
Convulsions	27	9 (33)	18 (67)	5.22 (1.82,16.7)	**0.003**
Sucking	200	153 (77)	47 (24)		
Not sucking	74	30 (41)	44 (59)	2.61 (1.26, 5.44)	**0.010**
Oxygen saturation ≥90%	179	130 (73)	49 (27)		
Oxygen saturation < 90%	83	46 (55)	37 (45)	2.34 (1.12, 4.96)	**0.025**
Cough≤ 1 day	195	126 (65)	69 (35)		
Cough > 1day	80	58 (73)	22 (28)	0.78 (0.49, 2.58)	0.53

OR =odds ratio; CI = confidence interval

Table 4

Table 4 Transcutaneous bilirubin levels at admission and the rise in bilirubin levels with benzylpenicillin +gentamicin and ceftriaxone

Serum bilirubin levels µmol/L	**Benzylpenicilln/ Gentamicin N**	**Benzylpenicillin/ Gentamcin requiring phototherapy N(%)**	**Ceftriaxone N**	**Ceftriaxone requiring Phototherapy N(%)**
<5	100	0	135	0
5 - <10	17	0	14	1
10 - <15	18	2	14	1
15 - <20	11	6	5	3
>20	14	11	2	2
Total	**130**	**19 (15%)**	**135**	**7 (6%) p=0.03**
Not done*	18	0	18	0
Received both antibiotic regimens*	17	1		
Rise in serum bilirubin level (µmol/L) during admission				
<10	26	0	24	0
>10	0	0	1	0
Total	**26**	**0**	**25**	**0**

***** 36 had no bilirubin measured or it was not measured immediately on admission

Table 5
Hearing test results (and neurological deficits) in survivors in the benzylpenicillin/gentamicin and ceftriaxone treatment arms at 6 months follow up

Hearing status(neuro deficits) N	Benzylpenicillin/gentamicin	Ceftriaxone	Received both antibiotics
Bilateral Hearing Loss	4 (4 with global delay)	2(1 CP 1 blind)	0
Unilateral Hearing Loss	5 (1 with global delay)	5	0
Total with hearing deifcts	**9**	**5**	**0**
Normal Hearing	50 (4 global delay, 1 hemiplegia (1 fine motor deficit)	52 (4 hydrocephalus, 2 blind (5 global delay, 1 hemiplegia)	8 (1 global delay)
TOTAL TESTED	**68 (11 with neuro deficits)**	**64 (14 with neuro deficits)**	**8 (1 with neuro deficits)**
Children not tested or Inconclusive result	56	61	4
TOTAL survivors at 6 months	**124**	**125**	**12**

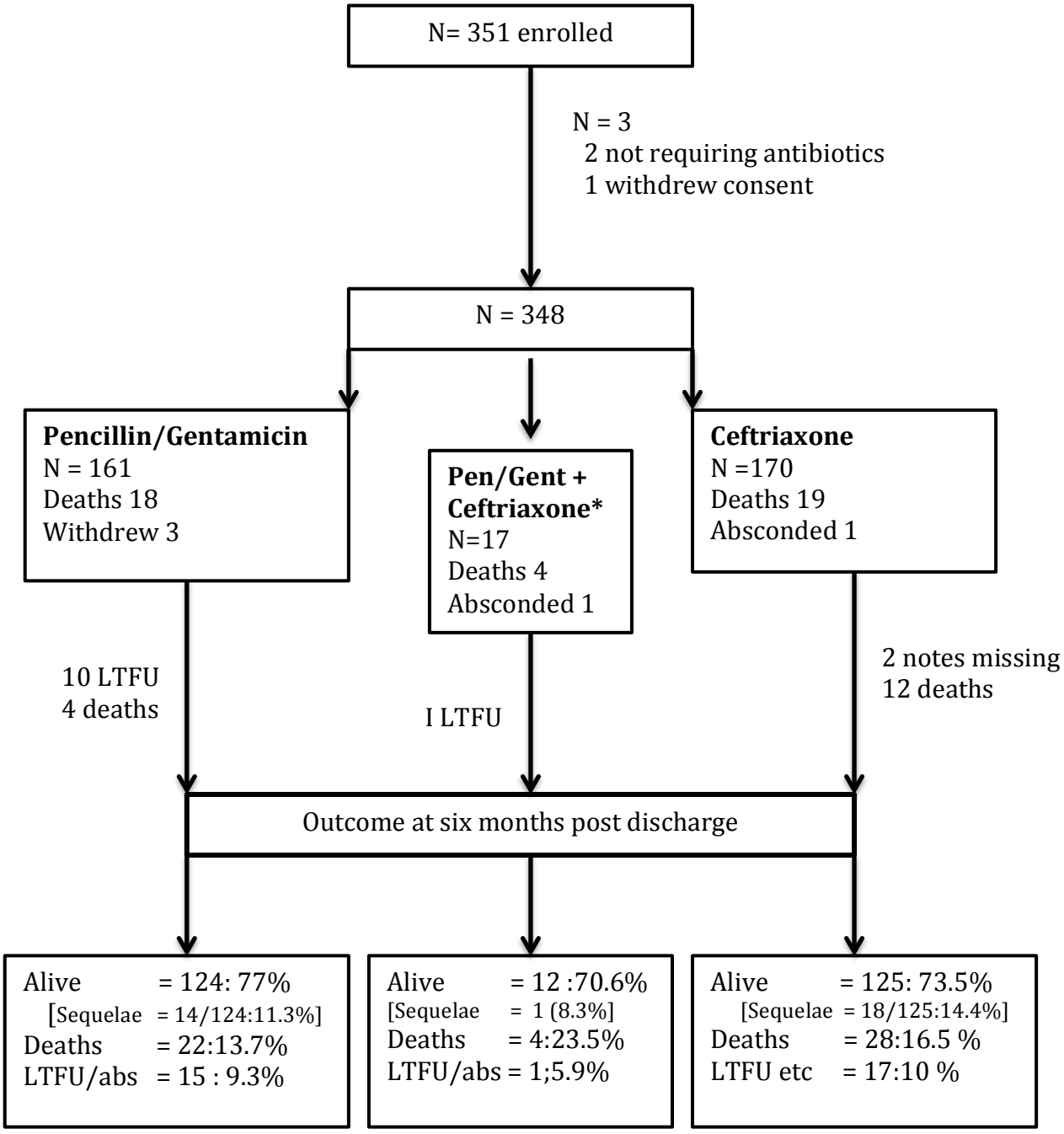
Table 7 ORIGINAL

Hearing test results and neurological deficits in treatment arms benzylpenicillin/gentamicin and ceftriaxone at 6 months follow up

Hearing status (neuro deficit)	N	benzylpenicillin/gentamicin	Ceftriaxone	received both antibiotics
Bilateral Hearing Loss	13	8(5 global delay)	4 (1 CP, 1 blind, 1 seizures)	1 (1 global delay)
Unilateral Hearing Loss	8	4(1 global delay)	4	0
Inconclusive test results	4	2	2	0
Normal Hearing	191	90 (4 global delay,) (1 hemiplegia 1 fine motor)	96 (4 hydrocephalus, 2 blind) (5 global delay, 1 hemiplegia)	5
TOTAL	**216**	**104 (12 neuro deficits)**	**106 (15 neuro deficits)**	**6 (1 neuro deficit)**
Not tested	135	62	67	6

neuro = neurological; CP = cerebral palsy

Figure 1

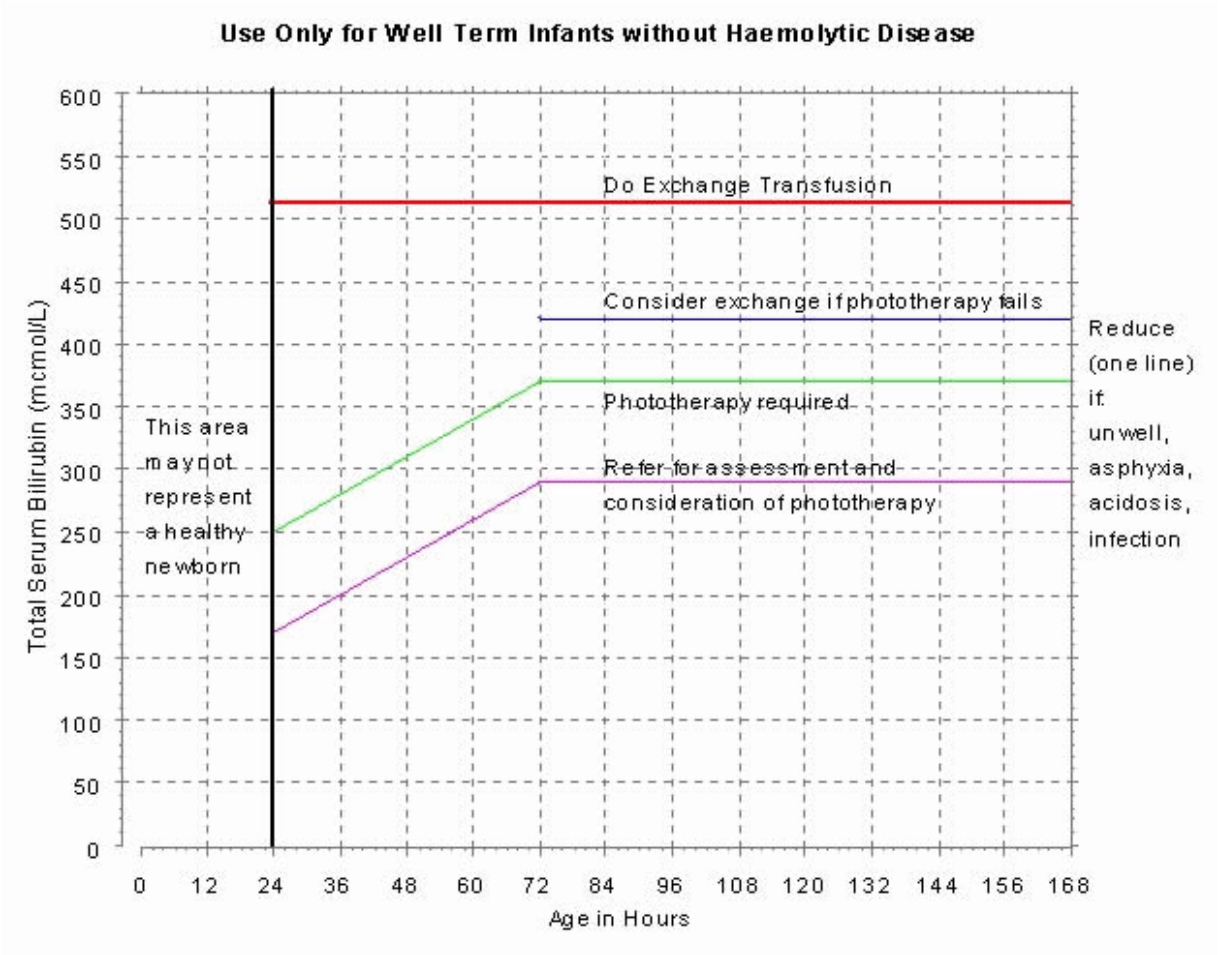


* ceftriaxone was added during treatment : LFTU = Lost to follow up: abs = absconded

Figure 1. Study enrollment and outcome

SDC Table 1

LEVELS OF BILIRUBIN AT WHICH TO START PHOTOTHERAPY IN TERM INFANTS



SDC Table 2

LEVELS OF BILIRUBIN AT WHICH TO START PHOTOTHERAPY IN PREMATURE INFANTS

Gestational age	Day 1	Day2	Day 3	Day 4	Day 5	Day 6	Day 7
24 wks	3.5 mg/dl	5.8	7.6	7.6	7.6	7.6	7.6
	60 umol/L	100	130	130	130	130	130
26 wks	5.3 mg/dl	7.6	9	9	9	9	9
	90 umol/L	130	160	160	160	160	160
28wks	6.4 mg/dl	8.8	10.5	10.5	10.5	10.5	10.5
	110 umol/L	150	180	180	180	180	180
30wks	8.2 mg/dl	10.5	12	12	12	12	12
	140 umol/L	180	210	21	210	210	210
32wks	9.3 mg/dl	11.7	13	13	13	13	13
	160 umol/L	200	230	230	230	230	230
34 wks	9.8 mg/dl	12.2	14	14	14	14	14
	170 umol/L	210	240	240	240	240	240
36 wks	11 mg/dl	13.4	15	15	15	15	15
	190umol/L	230	260	260	260	260	260

SDC Table 3 CSF and blood culture findings and outcomes in infants with possible severe bacterial infection.

3a CSF culture and overall outcome at 6 months post discharge

Culture	Outcome at 6 months post discharge					
	alive	dead	sequelae	LTFU*	Total	p value
No growth*	134	30	37	14	215	No growth v combined positive cultures P = 0.003 (Chi-squared test)
coagulase negative staphylococcus	4	3	0	0	7	
Group B streptococcus	6	3	3	0	12	
Not done	16	10	1	4	31	
Gram negatives	1	1	1	3	6	
Strep pneumoniae [a]	3	3	0	0	6	
Streptococcus pyogenes	2	0	0	0	2	
Total	**166**	**50**	**42**	**21**	**279**	

[a]*Streptococcus pneumonia*

Of 12 no growth but cell counts suggestive of Bacterial Meningitis, 4 were Alive, 1 Died, 1 had sequelae and 1 was lost to follow up *LFTU = lost to follow up/absconded

3b Blood culture and overall outcome at 6 months post discharge

Culture	Outcome at 6 months post discharge					
	alive	dead	sequelae	LTFU*	Total	p value
No growth	124	28	26	9	187	No growth v combined positive cultures P= 0.04
Coagulase neg staph[a]	30	7	8	7	52	
Group B streptococcus	5	5	1	1	12	
Not done	2	0	0	0	2	
Gram negatives	3	3	1	1	8	
Strep pneumoniae[b]	3	1	1	0	5	
A haem streptococcus	2	0	2	0	4	
Group D streptococcus	1	0	0	0	1	
Staphylococcus aureus	3	5	0	1	9	
Total	**172**	**49**	**39**	**19**	**279**	

*LFTU = lost to follow up [a] coagulase negative staphylococci; [b] *Streptococcus pneumonia;* [c] alpha haemolytic streptococcus.